Keeping Apples Disease-Free During Storage and Shipping

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Most inoculum of blue mold decay recycles from year to year on field bins. Good bin sanitation can keep inoculum levels low. If fruit must be treated with diphenylamine after harvest to control physiological storage disorders, then adding either Scholar or Penbotec to the drench solution will protect fruit from decay inoculum that collects in the drench solutions.

Blue mold and gray mold are the most common postharvest diseases of apples. Blue mold decays are soft, watery, and have a musty or earthy odor. The blue mold pathogen, *Penicillium expansum*, invades fruit wounds or fruit stems during long-term controlled atmosphere storage.

Fruit with gray mold often look like baked apples because they have a uniformly light tan skin, fairly firm flesh, and a cider-like odor. The gray mold pathogen, *Botrytis cinerea*, can invade fruit at wounds, but it also infects fruit in the field. In other crops, such as strawberries and kiwi, *B. cinerea* infects fruit during or shortly after bloom, then remains quiescent until fruit begin to ripen. Gray mold in stored apples often originates at the calyx end of fruit, a location that would be consistent with field infections of the calyx shortly after bloom.

When first introduced, the benzimidazole fungicides thiabendazole (Mertect 340F), benomyl (Benlate), and thiophanate-methyl (Topsin M) provided excellent control of both *P. expansum* and *B. cinerea* when applied in postharvest drenches. Even though benzimidazole-resistant strains of both pathogens emerged soon after these products were introduced in the 1970's, the benzimidazole fungicides continued to provide good control of blue mold decay until the early 1990's because the fungicides were almost always applied with diphenylamine (DPA). DPA suppressed benzimidazole-resistant strains of both pathogens. Gray mold can still be controlled using postharvest drenches that contain DPA plus thiabendazole (Mertect 340F). By the mid-1990's, however, blue mold had become a serious problem in some storages in New York State because *P. expansum* had developed resistance to the benzimidazole-DPA combination.

Epidemiological Studies

Studies were initiated in the mid-1990's to identify inoculum sources for *P. expansum* and sanitation methods that could be employed to reduce inoculum levels. Contaminated field bins were shown to carry huge quantities of inoculum from one season to the next, with some bins carrying more than 10^9 spores per bin (Table 1). Spores on contaminated bins are washed off the bins in autumn when the apples in the filled bins are given postharvest treatments using recycled drench solutions. Inoculum that accumulates in the drench solution causes decays, which can result in even dirtier bins being returned to the field the next season. Sanitizing bins has been shown to remove 99% of viable conidia, but few packinghouses routinely sanitize bins because of the cost involved and questions about the economic benefits of bin sanitation.

Although field bins were identified as a major inoculum source for *P. expansum*, the relative importance of recycled inoculum from field bins, as compared to "new" inoculum originating from the field each year, raised questions about the value of

TABLE 1

Numbers of viable *Penicillium* spores per bin that were released into wash water as determined by washing bins with a portable drencher and dilution-plating sub-samples from the wash water.

	Number of spores per bin recovered in wash water*
Summer 1999	
Group I non-sanitized oak bins	8.35×10^8
Group I following fresh sanitizer wash	1.54×10^6
Group I washed at the end of sanitizer usefulness	7.44×10^6
Group II non-sanitized oak bins from another CA room	4.25×10^8
Summer 2000	
Wooden bins (mixed oak and other wood)	2.23×10^9
Plastic bins from the same storage room	4.82×10^8

*Means were derived from washing five replicates of 5 bins each in 1999 and four replicates of five bins each in 2000. All of the non-sanitized bins contained dried-up decayed fruit that had been left in the bins as they were bundled at the end of the packing lines, and the decayed fruit present in the bins may have accounted for much of the inoculum.

bin sanitation. The effort to sanitize field bins might be wasted if large quantities of inoculum could be brought into the storage each year on apple surfaces or via soil stuck on the runners of field bins. Therefore, we initiated work to quantify populations of *P. expansum* that could be found in orchard soil and on apples at harvest time.

Quantifying *P. expansum* in orchard soils: Quantifying *P. expansum* populations in soil is difficult because of the diversity of organisms present in soils. To deal with this problem, we modified a selective medium described by Hocking and Pitt (1980) so that it could be used to isolate *P. expansum* from soil and from other environments that harbor a diverse microflora. The selective medium we used, DG18-P, does not prevent growth of other species of soil-inhabiting *Penicillium*, so quantifying *P. expansum* density in soils still required that colonies appearing on soil dilution plates be sub-sampled onto Czapek yeast-extract agar (CYA) where *P. expansum* could be readily identified due to its distinctive colony morphology and growth rate. Nevertheless, this selective medium provided a useful tool for quantifying inoculum from different sources.

Soils from five different apple orchards near Highland, NY, were sampled at various times during 2004 and 2005. In the four orchards that were being actively managed, soil was collected from the herbicided area beneath the tree canopy. The fifth orchard had been abandoned about 20 years ago and was largely overgrown with weeds, brambles and other shrubs. In each orchard, samples were collected from within the drip-line of five different trees that were separated by at least 10 meters. Soil was sampled by removing surface debris and/or cover plants with a shovel and then collecting approximately 50 cc of soil from the upper 8 cm of the soil profile at five different locations beneath each of the five sample trees. The five sub-samples from each tree were mixed together, but the bulked subsamples from each tree were evaluated separately to provide five replicate evaluations from each orchard.

Population densities of *Penicillium* species in the soils were determined by dilution plating on DG18-P agar. Three arbitrarily selected *Penicillium* colonies from each of ten soil-dilution plates were sub-cultured onto CYA plates for species identification. In a few cases where soil dilution plates had low numbers of colonies, more than three subcultures were taken from other plates in the same replicate to bring the total number of subcultures to 30 per replicate, or 150 per orchard. Benzimidazole resistance of all 150 subcultures per orchard was determined by stab-inoculating PDA amended with 5 ppm MBC (the fungicidal metabolite in benomyl and thiophanate-methyl). The relationship between the weight of the soil sampled and soil dry weight was determined by drying 4 grams of soil for 24 hours in a drying oven set at 100 °C. The ratio between original sample weight and dry weight was used to adjust counts so that the final concentration of *Penicillium* species in soil could be expressed as the number of colony-forming units (cfu) per gram of dry soil.

Densities of *P. expansum* in orchard soils ranged from 14 to 218 cfu/g of dry soil in the managed orchards but were roughly 10 times higher in the abandoned orchard (Orchard E, Table 2). Spore densities in orchard soils were surprisingly consistent from year to year in the four orchards soils that were evaluated in both 2004 and 2005.

We assumed that even in a worst-case scenario involving wet harvest weather with soil occasionally balled into the bin runners, bins would be unlikely to carry more than an average of 1 kg soil into drench solutions. Given that assumption, the contribution of orchard soils to build-up of *P. expansum* inoculum in postharvest treatment solutions is dwarfed by the inoculum that can originate with badly contaminated bins (Table 1). Contaminated bins can carry 10,000 times more inoculum than a kilogram of soil from the managed orchards that we tested and more than 1,000 times more inoculum than would be contained in a kilogram of soil from the abandoned orchard we tested.

Quantifying *P. expansum* on apple fruit at harvest: To determine how much inoculum may come into storages on the surface of harvested fruit, 10 arbitrarily selected apple fruits were harvested from each of three trees in four different orchards during the fall of 2005. One of the orchards was sampled on two different

TABLE 2

Results from sampling orchard soils in the Hudson Valley to determine populations of *P. expansum* and proportions of the populations that were benzimidazole-resistant.

Orchard	sampling date	cfu *Penicillium* species per g soil[z]		*P. expansum* as a percent of total *Penicillium* population	% *P. expansum* with benzimidazole resistance[x]	Estimated *P. expansum* spores per bin assuming 1 kg soil/bin
		all species	*P. expansum*[y]			
A	23-Jul-04	262	33	12.6	27	33,000
A	17-Jun-05	1008	218	21.6	24	218,000
B	3-Sep-04	3,440	182	5.3	0	182,000
B	17-Jun-05	1626	186	11.4	1	186,000
C	30-Jun-04	298	14	4.7	8	14,000
C	9-Jun-05	698	46	6.5	10	46,000
D	19-Jul-05	310	40	12.9	13	40,000
E	8-Sep-04	15,268	2,137	20.6	0	2,137,000
E	16-May-05	5,447	1,610	29.6	0	1,610,000

[z] The number of colony-forming units (cfu) was determined by dilution plating and is roughly equivalent to the number of spores present in the soil.

[y] The proportion of *P. expansum* present in the total population of *Penicillium* species was determined via sub-sampling onto Czapek yeast-extract agar.

[x] Incidence of benzimidazole resistance was determined by stab-inoculating agar plates amended with 5 ppm MBC.

TABLE 3

Results from washing apple fruit collected in Hudson Valley orchards to determine populations of *P. expansum* present on fruit surfaces at harvest.

Orchard	Variety/treatment	Sample date	Mean cfu/apple	No. of sub-cultures evaluated	% of total cfu that were *P. expansum*	Estimated *P. expansum* spores/bin of 2000 apples
A	Empire	21-Sep-05	52.0	540	28.3	29,467
A	Empire	**17-Oct-05	3.0	90	10.0	600
B	Rome Beauty	21-Oct-05	15.3	315	14.6	4,478
C	Golden Delicious	21-Sep-05	5.3	180	8.9	948
D	Delicious	21-Oct-05	20.7	315	19.7	8,135
HVL-1*	Honeycrisp	8-Sep-05	50.0	84	59.5	59,524
HVL-2	Honeycrisp	8-Sep-05	22.5	84	21.4	9,643
HVL-3	Honeycrisp	8-Sep-05	36.3	84	34.5	25,030

*Samples from Hudson Valley Lab research plots left unsprayed during summer (HVL-1) or sprayed with Topsin M + Captan (HVL-2) or Pristine (HVL-3) one day prior to sampling.

** Spore numbers were presumably reduced compared to earlier sampling in the same orchard due to 13.5 inches of rainfall that occurred 7-15 October.

dates. In addition, 10 apples were collected from each of four different Honeycrisp trees in replicated experimental plots that had received different summer fungicide regimes as part of a separate experiment at the Hudson Valley Lab. In all cases, fruit were brought to the lab where they were washed for 30 seconds in 500 ml of sterile distilled water containing 0.01% Tween 20. The wash water was then filtered to trap the *Penicillium* spores. The filter was subsequently washed in 5 ml of sterile distilled water and a sub-sample of that wash water was spread onto each of 5 plates of DG18-P agar. Plates were incubated at 25 °C for seven days, after which all visible colonies on the plates were counted. Varying numbers of arbitrarily selected colonies from each plate were sub-cultured onto CYA plates to determine what proportion of the *Penicillium* population on apple fruit consisted of *P. expansum*. Results were expressed as numbers of all *Penicillium* species per fruit and numbers of *P. expansum* per fruit (Table 3). The potential spore load for a full field bin was calculated by assuming that a field bin would hold approximately 2000 fruit.

The *P. expansum* populations on fruit surfaces ranged from a low of about 9 to a high of 28 cfu/fruit in the five samples taken from sprayed orchards (Orchards A-D, Table 1). In Orchard A, where fruit were collected from the same block of trees on both 21 September and again on 17 October, the significantly reduced population detected in the second sampling was probably attributable to the week of heavy rain that immediately preceded the second sample date. Empire apples were still available in this orchard on 17 October because some fruit damaged by hail during early summer were not harvested.

The number of *P. expansum* spores detected on Honeycrisp fruit in our fungicide trial was greatly affected by the fungicide treatments (Table 3). Fruit from control trees that received their last fungicide spray (Topsin M 11 oz/A+ Ziram Granuflo 4 lb/A) on 19 July had more than twice as many *P. expansum* spores as fruit that were sprayed with Pristine (4.8 oz/100 gal) the day prior to harvest. Trees treated with Topsin M 4 oz/100 gal plus Captan 80WDG 10 oz/100 gal on the day prior to harvest had only one-sixth as many *P. expansum* spores as control trees (Table 3). Nearly 60% of all *Penicillium* spores on apples from control trees turned out to be *P. expansum*, whereas only 21% and 35% of the *Penicillium* spores on fruit from the Topsin M/Captan and Pristine treatments, respectively, were *P. expansum*. Those results suggest that the fungicides may be more effective against *P. expansum* than against some of the other *Penicillium* species that are present in orchards. Based on our limited sampling in 2005, the numbers of spores that might be brought into storage on fruit surfaces is dwarfed by the inoculum previously measured on contaminated field bins (Table 3).

The accumulated evidence from measuring *P. expansum* populations on field bins, in orchard soils, and on apple fruit at harvest suggests that badly contaminated field bins are by far the most important potential source of inoculum for *P. expansum* under conditions prevalent in New York State. In the absence of effective fungicides, sanitizing contaminated field bins should reduce losses to blue mold decay in storages where decay has gradually increased from year to year. Where storage operators choose to use one of the new fungicides (pyrimethanil or fludioxonil) to control *P. expansum*, bin sanitation should still be used to reduce selection pressure for resistance to these new fungicides. It may not be cost effective to sanitize all bins every year, but badly contaminated bins (i.e., those showing visible blue stains from fruit that had blue mold decay) should always be sanitized before they are returned to the orchard for refilling.

Postharvest Fungicide Options

The best option for minimizing blue mold decay in stored fruit involves using clean bins, avoiding drenches after harvest, and storing apples in sanitized storage rooms. Sanitation alone can significantly reduce the incidence of decay as illustrated by the experimental results from inoculations with varying inoculum densities (Table 4). However, postharvest treatment with diphenylamine (DPA) may be needed to control storage scald and/or carbon dioxide injury with some cultivars. Postharvest fungicide treatment may also be desired to control gray mold decay caused by *Botrytis cinerea*. When fruit are moved into storage without a postharvest treatment, the incidence of blue mold is usually low, but gray mold sometimes emerges as a problem.

Thiabendazole (trade name: Mertect 340F) and captan are still registered for postharvest treatment of apples. Captan is usually used in combination with Mertect 340F. Many storage operators report that the combination of Mertect 340F and captan is more effective than Mertect 340F used alone. However, in repeated

TABLE 4

Effects of inoculum density and 1-MCP treatment on incidence of decays caused by *P. expansum* following wound inoculations at harvest in 2004 and 2005.

Inoculum density: Number of spores/wound:	% fruit with decay				Grand means for effects of inoculum density
	No 1-MCP applied		With 1-MCP applied		
	2004 63 days	2005 60 days	2004 63 days	2005 60 days	
5	9 a[z]	48 a	13 a	72 a	40 a
25	23 b	64 b	27 b	89 b	56 b
100	36 c	80 c	43 c	94 c	67 c
Grand means: 1-MCP effects[x]	**44 A**		**56 B**		

[z] Means within columns followed by the same small letter are not significantly different ($P \leq 0.05$) as determined using Fisher's Protected LSD to separate means from the two-way analyses.

[x] The grand means for effect of 1-MCP treatment represent the means of all three inoculum concentrations for both years of the experiment and were significantly different ($P \leq 0.05$).

testing where wounded fruit were inoculated just prior to application of fungicides, captan has always been less effective for protecting fruit than are Penbotec, Scholar, and (in the absence of resistance) Mertect 340F. The difference between perceived effectiveness of captan in commercial operations as compared to controlled trials may be related to the way captan works. Captan may kill spores that accumulate in drench solutions, thereby decreasing inoculum availability and reducing fruit infection, even though it performs poorly in controlled tests where inoculum is applied to fruit just before or after captan treatment. That hypothesis is currently being tested.

Two new fungicides were recently registered for postharvest treatment of apples in the U.S. Pyrimethanil (trade name: Penbotec) and fludioxonil (trade name: Scholar) are extremely effective for controlling blue mold and gray mold on apples. Both Penbotec and Scholar are fully compatible with DPA and calcium chloride. Both products are very stable and hold up well in postharvest drench solutions. Both products are registered for use in drenches as well as for application in packinghouse line sprays. The line spray application should reduce chances that decays will develop in packed fruit after it enters distribution channels.

Packinghouse operators choosing to use these new fungicides should use Penbotec in one year and Scholar the next year so that *Penicillium* spores that recycle on bins will not be repeatedly exposed to the same fungicide year after year. Penbotec and Scholar have different modes of action, and both of them are distinctly different from Mertect 340F. Alternating annually between Penbotec and Scholar should reduce selection pressure for resistance to both of these new fungicide chemistries. Adding captan to the Penbotec or Scholar in drench solutions might further reduce selection pressure for resistant isolates, but that strategy needs further testing before it can be recommended. Alternation of chemistries for fungicides applied in packinghouse line sprays is of less concern because the treated fruit are moved into the retail supply chain before any surviving infections can sporulate, thereby reducing or eliminating selection for fungicide resistance.

Although Scholar and Penbotec are approved for use in the United States, residue tolerances for these fungicides have not yet been established in some apple-importing countries. Before applying these fungicides to apples destined for export, packinghouse operators should verify that the importing country will accept product treated with the fungicide in question. A database of approved MRLs (maximum residue levels) for various commodities and countries can be found at the following website: http://mrldatabase.com.

Effect of 1-MCP on Postharvest Decays

Studies were conducted in NY to determine if 1-MCP makes apples more susceptible to postharvest decays, but our results were not always consistent with results from researchers in other areas. In experiments we conducted during the 2003-

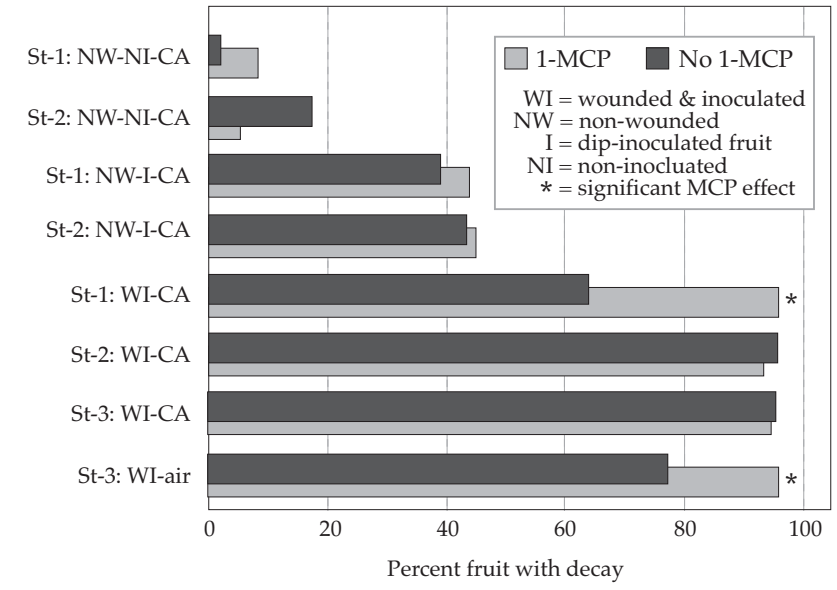

Figure 1. Effects of 1-MCP treatment on incidence of decay in experiments initiated in fall of 2003. Treated and non-treated fruit were held in three different CA storages, and a comparable sample was held in air storage at St-3. None of the fruit in these experiments received any postharvest fungicide treatment.

TABLE 5

Effects of fungicide and 1-MCP treatments on fruit firmness and on development of blue mold decay in wound-inoculated Empire apples that were stored in cold air for 90 days at 36°F.

Material and rate of formulated product per 100 gal drench solution	% fruit with blue mold		Mean fruit firmness (lb)	
	With 1-MCP	No 1-MCP	With 1-MCP	No 1-MCP
Control	100*b*	94 b	11.1 a	9.1 a
Mertect 340F 16 fl oz	100*b	86 b	11.3 a	9 a
Scholar 50W 8 oz	0 a	1 a	11.3 a	9.3 a
Penbotec 40% 16 fl oz	0 a	0 a	11.3 a	9.5 a
Grand means: effects of 1-MCP			**11.2 B**	**9.3 A**

*Means within columns followed by the same small letter are not significantly different ($P \leq 0.05$) as determined using Fisher's Protected LSD to separate means from the two-way analyses. Means followed by asterisks indicate significant differences between simple means for fruit with/without 1-MCP treatment.

2004 storage season, fruit treated with 1-MCP developed more decay than non-treated fruit in one of seven observations involving fruit held in three different CA storages, but treatment with 1-MCP had no statistically significant effect on decay development in the other six CA trials (Figure 1). However, in one trial in 2003 and in a number of subsequent experiments, inoculated fruit held in cold-air storage developed more decay when treated with 1-MCP than did non-treated fruit.

In an experiment conducted at the Hudson Valley Lab in 2004 and repeated in 2005, wounded Empire apples were inoculated with *P. expansum* using three different inoculum densities. Some fruit were treated with 1-MCP and other fruit were stored without treatment. All of the fruit were held in cold-air storage at 36° F for approximately nine weeks. Incidence of decay was consistently higher in fruit treated with 1-MCP than in non-treated fruit (Table 4).

Why have our trials and those of other researchers provided inconsistent results concerning effects of 1-MCP on decay incidence? We suspect that results vary depending on storage conditions (CA or cold-air) and varying delays between the application of 1-MCP and the establishment of the CA atmospheres. Both 1-MCP treatment and low-oxygen storage conditions may disable the natural defense mechanisms that allow stored apples to defend themselves against infections by

inoculated and fungicide treatments were applied, half of the wounded and inoculated apples and half of the non-wounded, non-inoculated apples from each treatment (i.e., 20 fruit per replicate) were exposed to 1 ppm 1-MCP. All fruit were then moved to a 36°F cold room where they were held until 3 January. Inoculated fruit were observed for decay development after 90 days of cold storage. Firmness of the non-wounded, non-inoculated apples was determined at the same time using 20 fruit from each of four replicates and testing opposite sides of each fruit.

Mertect failed to control decay because the inoculum included resistant isolates, but the other fungicides provided excellent decay control through 90 days of cold storage (Table 5). Fruit treated with 1-MCP were significantly firmer than those not receiving 1-MCP treatment, but none of the fungicide treatments had any effect on fruit firmness. This experiment clearly showed that even under high-inoculum conditions, 1-MCP treatment will not adversely affect fungicide performance, nor will fungicides interfere with the effectiveness of 1-MCP treatments.

The introduction of 1-MCP may have helped to reduce losses to decay during long-term storage by delaying fruit senescence and allowing fruit to arrive in stores in better condition. Grocery store surveys have shown that the incidence of decays in fruit in poly bags has declined since 1-MCP was introduced. It is impossible to determine if the reduction of decay in bagged fruit in grocery stores is attributable to effects of 1-MCP, or to other factors such as improved packinghouse sanitation. It is most likely that a combination of factors is involved for the improved quality noted in grocery stores over the past several years.

P. expansum. When apples are held in cold-air storage, 1-MCP treatment may slow or arrest the normal wound-healing response in the fruit, but low-oxygen conditions in CA storage probably have the same effect. As a result, comparisons of decay development in inoculated fruit would show an effect of 1-MCP treatment in cold-air storage, whereas that effect might be masked in CA storages.

Even if treatment with 1-MCP slightly alters the susceptibility of apples to postharvest infections, another experiment showed that such differences are not detectable if fruit are treated with effective postharvest fungicides. For that experiment, uniformly wounded Empire apples were immersed for 30 seconds in a suspension containing 10,000 spores/ml of *P. expansum*, with half of the spores from a benzimidazole-sensitive isolate and the other half from a benzimidazole-resistant isolate. Fruit were then immersed for 30 seconds in either water or fungicide suspensions. Fungicide treatments were similarly applied to equal numbers of non-wounded, non-inoculated fruit that were used for firmness evaluations at the end of the experiment. The day after fruit were

Summary

Studies on inoculum cycling of *Penicillium expansum*, the cause of blue mold decay in stored apples, has shown that most inoculum recycles from year to year on field bins. Proper bin sanitation can keep inoculum levels low, and the incidence of decays in wounded fruit declines as inoculum levels are reduced. If fruit must be treated with diphenylamine after harvest to control physiological storage disorders, then adding either Scholar or Penbotec to the drench solution will protect fruit from decay inoculum that collects in the drench solutions. Although treatment with 1-MCP can make fruit more susceptible to decay when fruit are held in air storage, 1-MCP treatment does not appear to affect decay incidence in CA storages.

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